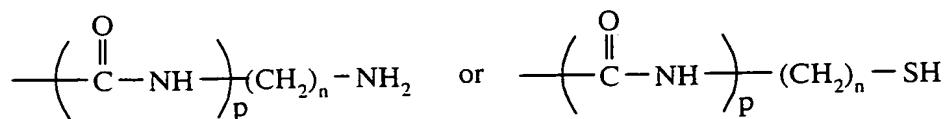
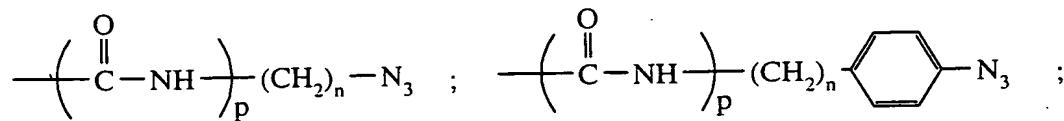
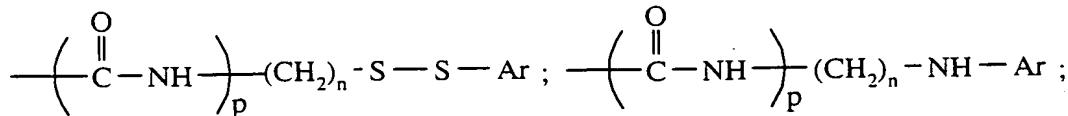
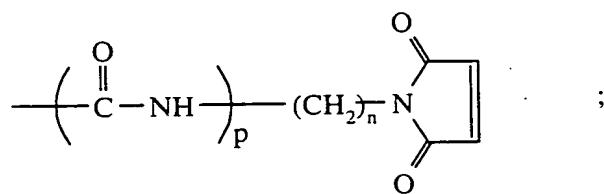
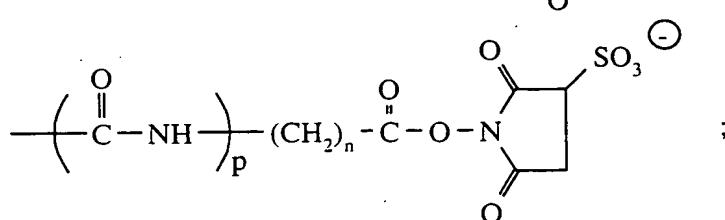
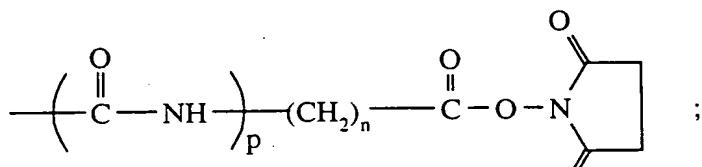
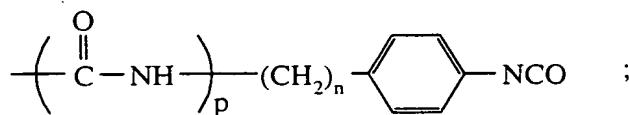
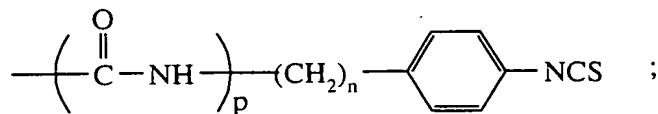
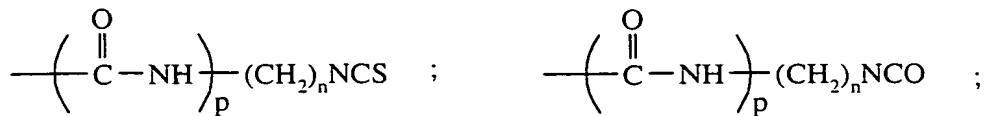


CLAIMS

1. A fluorescent entity comprising a fluorophore, with the exception of a rare earth metal cryptate, covalently attached to one or more oligonucleotide(s) or oligonucleotide analog(s), characterized in that it comprises at least one functional group, introduced or generated on the fluorophore or one of the oligonucleotides or oligonucleotide analogs.
- 10 2. The entity as claimed in claim 1, characterized in that the oligonucleotide or the oligonucleotide analog comprises from 2 to 60 nucleotide units.
- 15 3. The entity as claimed in claim 1 or 2, characterized in that the functional group can be attached to said entity via a spacer arm.
- 20 4. The entity as claimed in any one of claims 1 to 3, characterized in that the fluorophore comprises one or more aromatic rings and has a high molecular extinction coefficient, greater than 20 000, preferably greater than 50 000.
- 25 5. The entity as claimed in one of claims 1 to 4, characterized in that the fluorophore is chosen from rhodamines, cyanins, squaraines, bodipys, fluoresceines and their derivatives.
- 30 6. The entity as claimed in any one of claims 1 to 5, characterized in that the functional group is chosen from the groups: maleimide, carboxylic acid, haloacetamide, alkyl halide, azido, hydrazido, aldehyde, ketone, amino, sulfhydryl, isothiocyanate, isocyanate, monochlorotriazine, dichlorotriazine, aziridine, sulfonyl halide, acid halide, hydroxysuccinimide ester,
- 35

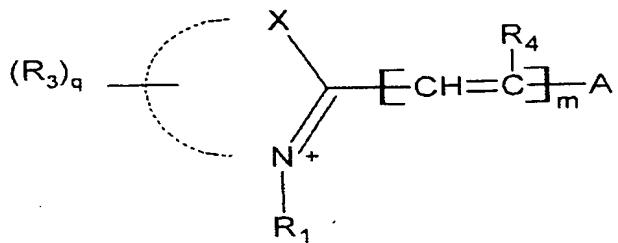
hydroxysulfosuccinimide ester, imido ester, hydrazide, azidonitrophenyl, azidophenyl, azide, 3-(2-pyridyldithio)propionamide and glyoxal, and more particularly the groups of formula:



where n ranges from 0 to 8 and p is equal to 0 or 1, and Ar is a 5- or 6-membered heterocycle comprising 1 to 3 hetero atoms, optionally substituted with a halogen atom.

5

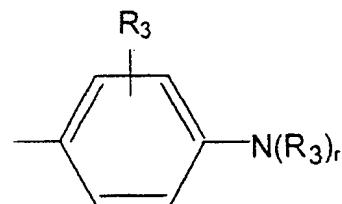
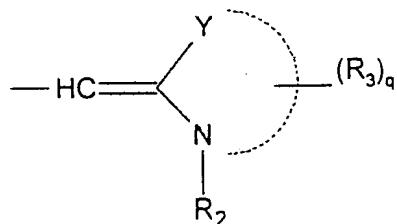
7. The fluorescent entity as claimed in any one of claims 1 to 6, of formula (I):



(I)

10 in which:

- A represents a group chosen from:



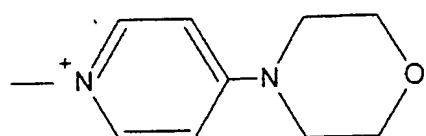
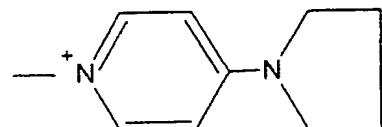
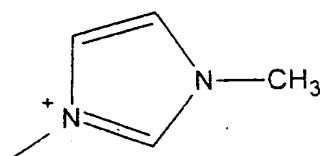
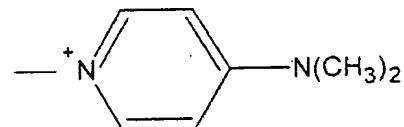
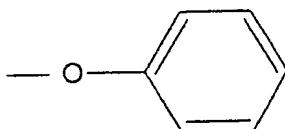
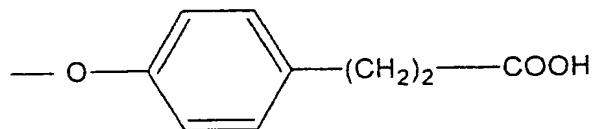
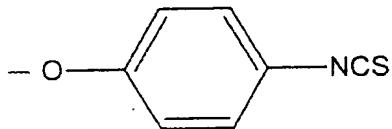
r = 2 or 3

— N(R3)r

r = 2 or 3

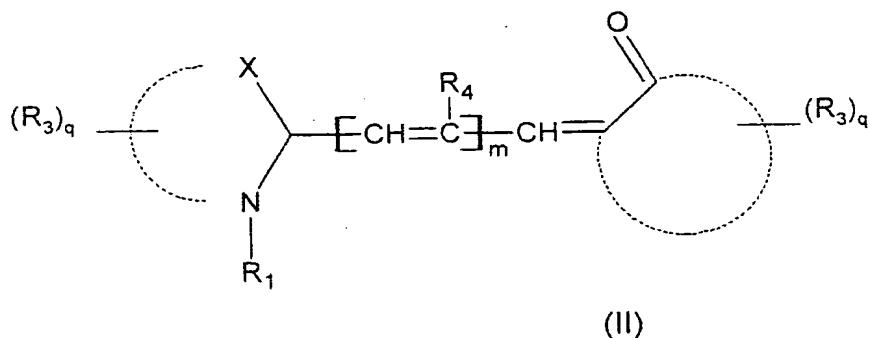
15 - the dashed lines each represent the carbon atoms required to form 1 to 3 fused rings, the groups R3 being attached to these rings;

- X and Y each represent N, C=O, O, S or C(CH<sub>3</sub>)<sub>2</sub>
- m has a value 1, 2, 3 or 4;
- q has a value 1, 2 or 3;
- (R<sub>3</sub>)<sub>q</sub> represents q groups R<sub>3</sub>, which may be identical
- 5 or different;
- the groups R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are identical or different and are chosen from hydrogen; a group -(CH<sub>2</sub>)<sub>s</sub>-Z in which s ranges from 0 to 4 and Z represents a group CH<sub>3</sub>, SO<sub>3</sub>H, OH or N<sup>+</sup>R<sub>1</sub>R<sub>2</sub>R<sub>3</sub> in which R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are as
- 10 defined above; a functional group as defined in claim 6; and an oligonucleotide or oligonucleotide analog optionally comprising a functional group as defined in claim 6;
- R<sub>4</sub> is chosen from: H; OH; CH<sub>3</sub>; Cl and the groups of
- 15 formula:



5 the substituents  $R_4$  in the allylic position possibly forming, with the polyethylenic chain, 1 to 3 fused rings containing from 4 to 14 atoms, which may or may not be saturated, said rings possibly containing one or more atoms of O, N and S, and possibly being optionally substituted with an oxo group.

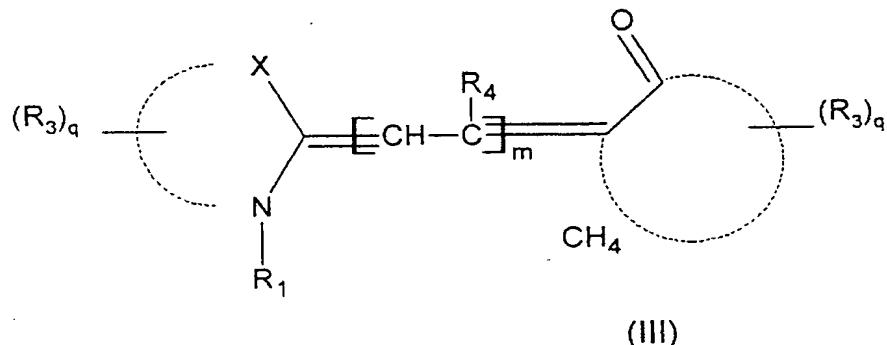
8. The fluorescent entity as claimed in any one of claims 1 to 6, of formula (II) or (III):



(II)

10

or



(III)

in which

15 - the dashed lines each represent the carbon atoms required to form 1 to 3 fused rings, the groups  $R_3$  being attached to these rings;

-  $X$  represents N, C=O, O, S or  $C(CH_3)_2$ ;

-  $m$  has a value 1, 2, 3 or 4;

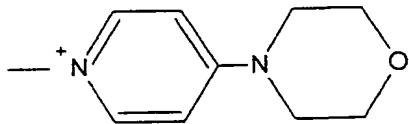
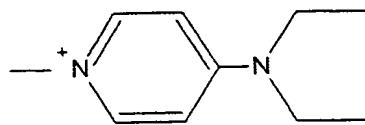
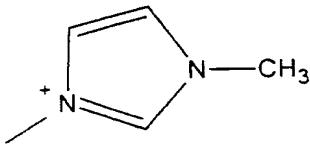
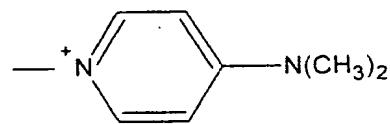
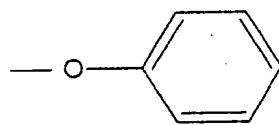
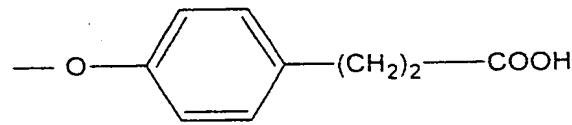
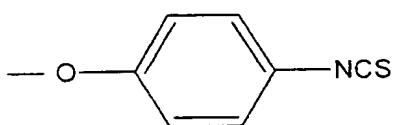
-  $q$  has a value 1, 2 or 3;

20 -  $(R_3)_q$  represents  $q$  groups  $R_3$ , which may be identical or different;

- the groups  $R_1$  and  $R_3$ , which may be identical or different, are chosen from hydrogen; a group  $-(CH_2)_s-Z$  in which  $s$  ranges from 0 to 4 and  $Z$  represents a group  $CH_3$ ,  $SO_3H$ ,  $OH$  or  $N^+R_1R_2R_3$  in which  $R_1$ ,  $R_2$  and  $R_3$  are as defined above; a functional group as defined in claim 6; an oligonucleotide or oligonucleotide analog optionally comprising a functional group as defined in claim 6;

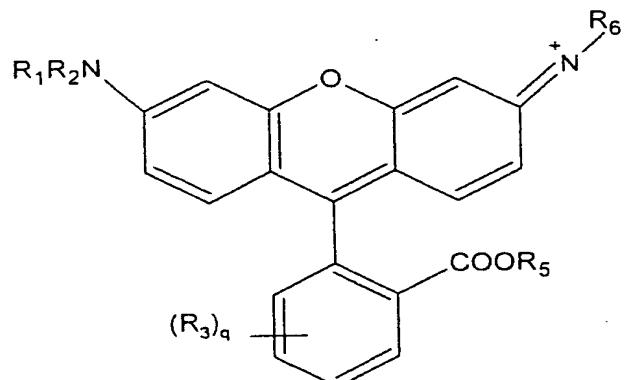
5 -  $R_4$  is chosen from:  $H$ ;  $OH$ ;  $CH_3$ ;  $Cl$  and the groups of formula:

10

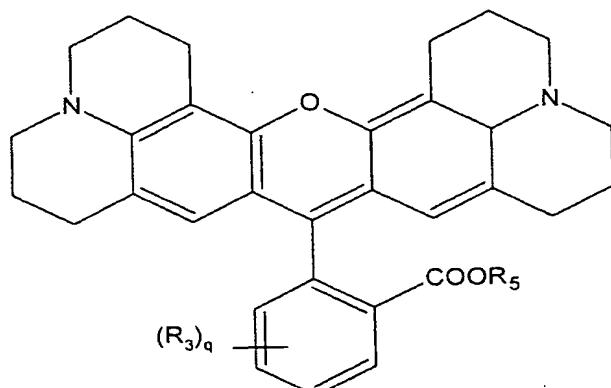


15 the substituents  $R_4$  in the allylic position possibly forming, with the polyethylenic chain, 1 to 3 fused rings containing from 4 to 14 atoms, which may or may not be saturated, said rings possibly containing one or more atoms of O, N and S, and possibly being optionally substituted with an oxo group.

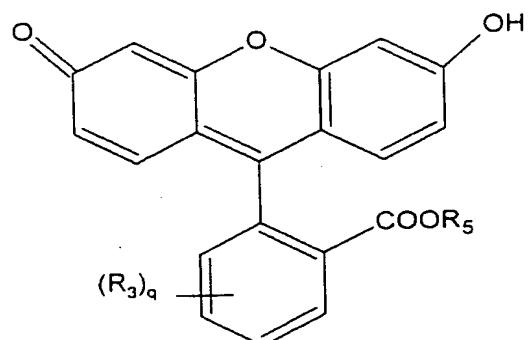
9. The fluorescent entity as claimed in any one of claims 1 to 6, of formula (IV), (V), (VI) or (VII):



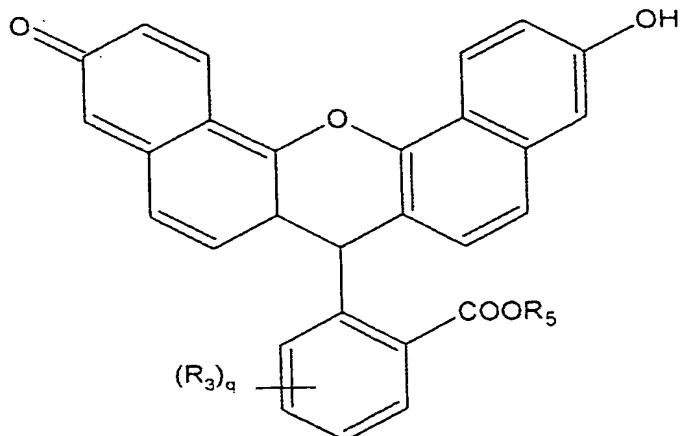
(IV)



(V)



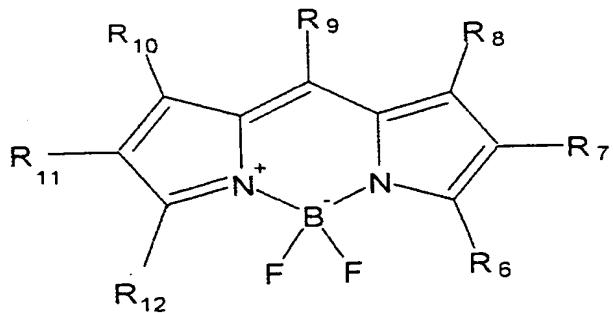
(VI)



(VII)

in which  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_5$  are identical or different  
5 and are chosen from hydrogen; a group  $-(CH_2)_s-Z$  in  
which  $s$  ranges from 0 to 4 and  $Z$  represents a group  
 $CH_3$ ,  $SO_3H$ ,  $OH$  or  $N^+R_1R_2R_3$  in which  $R_1$ ,  $R_2$  and  $R_3$  are as  
defined above; a functional group as defined in  
claim 6; and an oligonucleotide or oligonucleotide  
10 analog optionally comprising a functional group as  
defined in claim 6;  
-  $q$  has a value 1, 2 or 3.

10. The fluorescent entity as claimed in any one of  
15 claims 1 to 6, of formula (VIII):

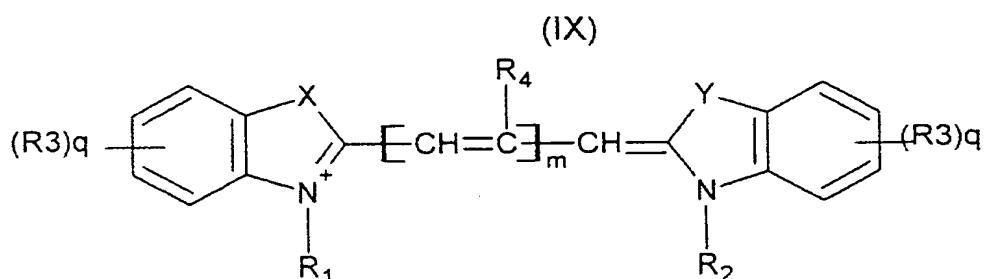


(VIII)

in which the substituents R<sub>6</sub> to R<sub>12</sub> are chosen from:  
hydrogen; a halogen; an alkyl; a cycloalkyl; aryl;  
arylalkyl; acyl; sulfo; a functional group as defined in  
5 claim 6; and an oligonucleotide or oligonucleotide analog  
optionally comprising a functional group chosen from  
those mentioned in claim 6.

11. The entity as claimed in claim 7, of formula (IX):

10



in which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, X, Y, m and q are as defined  
above.

15

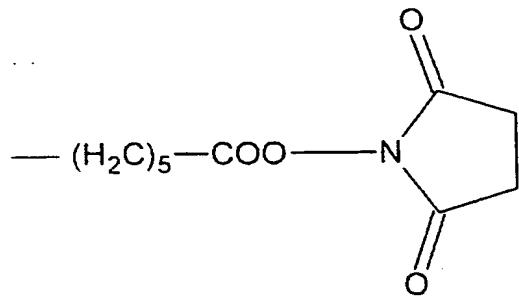
12. The entity as claimed in claim 11, in which X and Y  
each represent a group C(CH<sub>3</sub>)<sub>2</sub>.

13. The entity as claimed in claim 11, in which

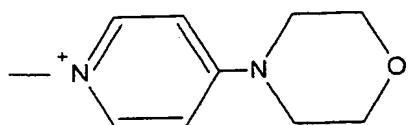
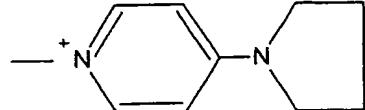
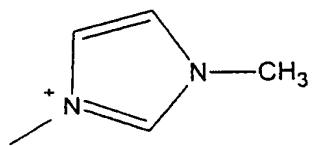
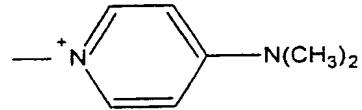
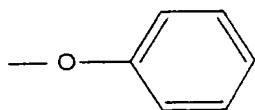
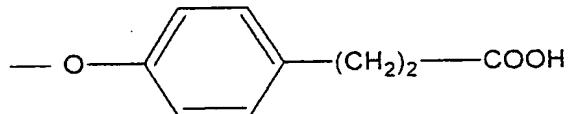
20

- R<sub>1</sub> and R<sub>2</sub> represent an alkyl comprising from 1 to 4  
carbon atoms or a group of formula below, at least  
one of the groups R<sub>1</sub> and R<sub>2</sub> representing a group of  
formula below:

25



- $R_4$  represents hydrogen
- $q = 1, m = 2$
- 5 -  $R_3$  represents hydrogen; a group  $-(CH_2)_s-Z$  in which  $s$  ranges from 0 to 4 and  $Z$  represents a group  $CH_3$ ,  $SO_3H$ ,  $OH$  or  $N^+R_1R_2R_3$  in which  $R_1$ ,  $R_2$  and  $R_3$  are as defined above; a functional group as defined in claim 6; an oligonucleotide or oligonucleotide analog optionally comprising a functional group as defined in claim 6;
- 10 -  $R_4$  is chosen from:  $H$ ;  $OH$ ;  $CH_3$ ;  $Cl$  and the groups of formula:

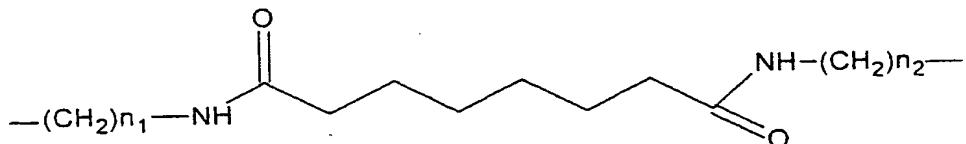
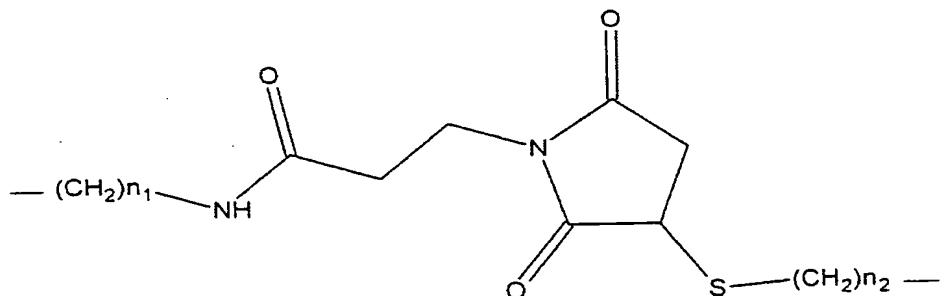
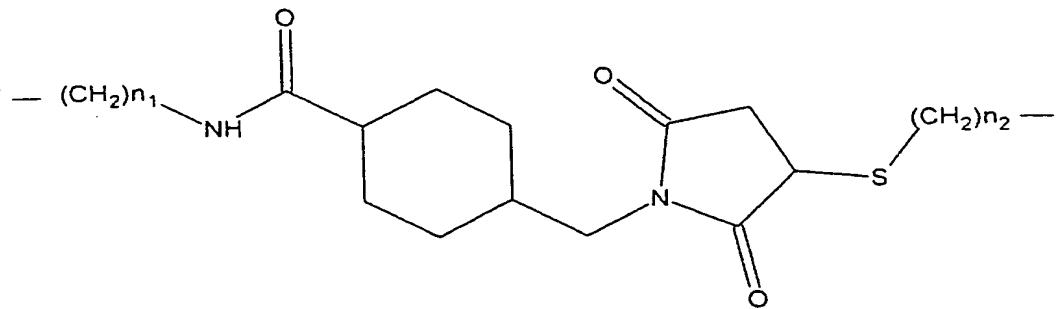


the substituents  $R_4$  in the allylic position possibly forming, with the polyethylenic chain, 1 to 3 fused rings containing from 4 to 14 atoms, which may or may not be 5 saturated, said rings possibly containing one or more atoms of O, N and S, and possibly being optionally substituted with an oxo group.

14. The entity as claimed in any one of claims 1 to 13, 10 characterized in that the fluorophore is covalently attached to the oligonucleotide either directly or via a spacer arm.

15. The entity as claimed in claim 14, characterized in that the fluorophore is attached to the oligonucleotide via a spacer arm consisting of a divalent organic radical chosen from linear or branched  $C_1-C_{20}$  alkylene groups optionally containing one or more double bonds or triple 20 bonds and/or optionally containing one or more hetero atoms, such as oxygen, nitrogen, sulfur, phosphorus, or one or more carbamoyl or carboxamido group(s);  $C_5-C_8$  cycloalkylene groups and  $C_6-C_{14}$  arylene groups, said alkylene, cycloalkylene or arylene groups optionally being substituted with alkyl, aryl or sulfonate groups.

25 16. The entity as claimed in claim 15, characterized in that the spacer arm is chosen from the groups:



in which  $n_1$  and  $n_2$  are between 2 and 6.

5 17. The entity as claimed in any one of claims 1 to 16,  
characterized in that the oligonucleotide comprises from  
5 to 60, in particular 5 to 20, preferably from 5 to 15,  
nucleotide units.

10 18. The entity as claimed in claim 17, characterized in  
that the oligonucleotide consists of a series of  
ribonucleotide or deoxyribonucleotide units attached to  
one another via bonds of the phosphodiester type.

19. The entity as claimed in claim 17, characterized in that the oligonucleotide consists of a series of ribonucleotide or deoxyribonucleotide units or of nucleotide analog units modified on the sugar or on the 5 base, attached to one another by natural internucleotide bonds of the phosphodiester type, some of the internucleotide bonds being optionally replaced with phosphonate, phosphoramido or phosphorothioate bonds.

10 20. The entity as claimed in claim 17, characterized in that the oligonucleotide consists of a series comprising both ribonucleotide or deoxyribonucleotide units attached to one another by bonds of the phosphodiester type and nucleoside analog units attached to one another by amide 15 bonds.

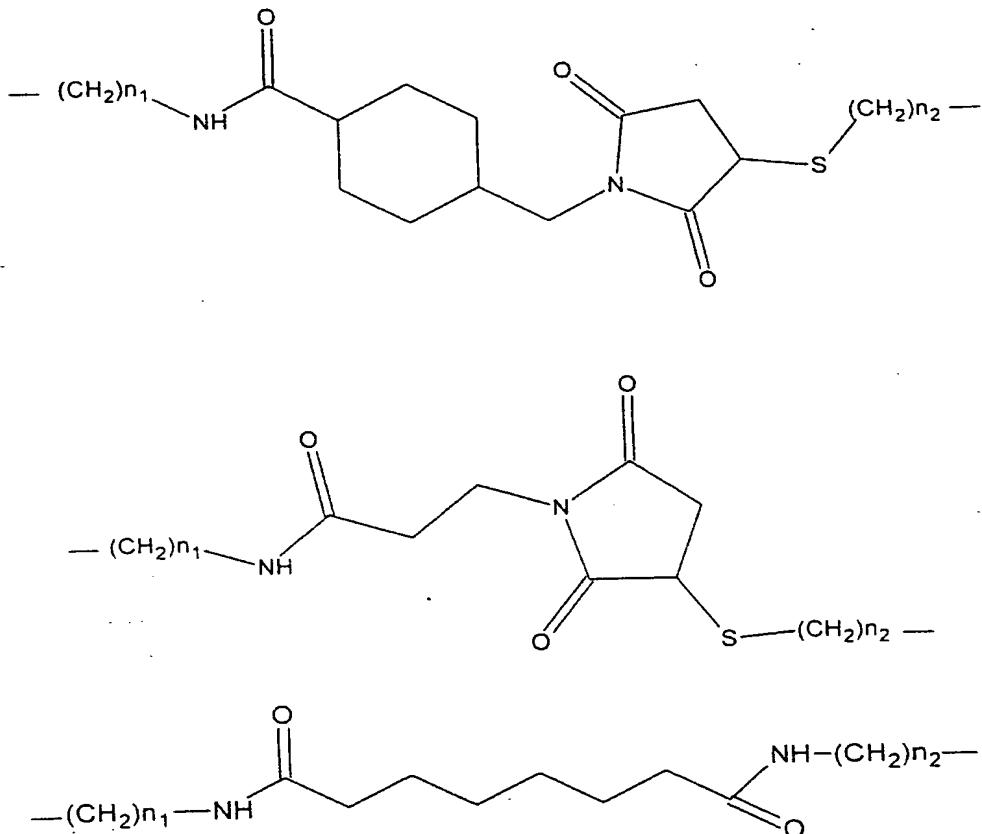
21. The entity as claimed in claim 17, characterized in that the oligonucleotide consists of a series of ribonucleotide or deoxyribonucleotide units attached to 20 one another by bonds of the phosphodiester type and of nucleoside analog units attached to one another by amide bonds, said oligonucleotide comprising at least 5 internucleotide bonds of the phosphodiester type at the end intended to be attached to the fluorophore.

25 22. The entity as claimed in any one of claims 1 to 21, characterized in that the functional group is an amine function of a nucleotide unit of the oligonucleotide or of the oligonucleotide analog, or results from the 30 reaction of a free amine function of a nucleotide unit of the oligonucleotide or the oligonucleotide analog, with a group chosen from the groups: ester, carboxylic acid, isothiocyanate, aldehyde, carbonyl, sulfonyl halide, alkyl halide, azide, hydrazide, dichlorotriazine, 35 anhydride, haloacetamide, maleimide and sulphydryl.

23. The entity as claimed in any one of claims 1 to 22, characterized in that the functional group results from the reaction of a free amine function of a nucleotide unit of the oligonucleotide or of the oligonucleotide 5 analog, with an N-hydroxysuccinimidyl ester.

24. The entity as claimed in claims 1 to 23, characterized in that the functional group(s) is (are) attached to the fluorophore and/or to the oligonucleotide 10 by a spacer arm consisting of a divalent organic radical, chosen from linear or branched C<sub>1</sub>-C<sub>20</sub> alkylene groups optionally containing one or more double bonds or triple bonds and/or optionally containing one or more hetero atoms, such as oxygen, nitrogen, sulfur, phosphorus, or 15 one or more carbamoyl or carboxamido group(s); C<sub>5</sub>-C<sub>8</sub> cycloalkylene groups and C<sub>6</sub>-C<sub>14</sub> arylene groups, said alkylene, cycloalkylene or arylene groups being optionally substituted with alkyl, aryl or sulfonate groups.

20 25. The entity as claimed in claim 24, characterized in that the spacer arm is chosen from the groups:



in which  $n_1$  and  $n_2$  are between 2 and 6.

5

26. A fluorescent conjugate consisting of an entity as claimed in any one of claims 1 to 25 covalently attached to a carrier molecule.

10 27. The conjugate as claimed in claim 26, characterized in that the fluorophore of the fluorescent entity is cyanin-5, the oligonucleotide of said entity has the sequence A<sub>15</sub> and the carrier molecule is cAMP.

15 28. The conjugate as claimed in either of claims 26 and 27, characterized in that the final molar ratio is greater than 0 and less than 100, preferably greater than 0 and less than 20.

29. The fluorescent conjugate as claimed in any one of claims 26 to 28, characterized in that the carrier molecule is an antibody, an antigen, an intracellular messenger, an intercellular messenger, a protein, a 5 peptide, a hapten, a lectin, biotin, avidin, streptavidin, a toxin, a carbohydrate, an oligosaccharide, a polysaccharide, a nucleic acid, a hormone, a vitamin, a medicinal product, a polymer, a polymeric particle, glass, a particle of glass or a 10 surface made of glass or of a polymer.

30. The fluorescent conjugate as claimed in claim 29, characterized in that the carrier molecule is an antibody or streptavidin.

15 31. The use of a fluorescent entity as claimed in any one of claims 1 to 25, or of a fluorescent conjugate as claimed in any one of claims 26 to 30, as a fluorescent tracer.

20 32. The use as claimed in claim 31, for detecting and/or determining, by fluorescence, an analyte in a medium liable to contain it.

25 33. The use as claimed in claim 32, for determining an interaction between biomolecules; or for determining a biological activity such as: an enzyme activity, the activation of a membrane-bound receptor, the transcription of a gene, a membrane transport or a 30 variation in membrane polarization.

34. The use as claimed in claims 31 to 33, in a method for screening medicinal products.

35 35. The use as claimed in claim 34, in which a fluorescent conjugate as claimed in any one of claims 26

to 30 is used as an acceptor fluorescent compound in the presence of a donor fluorescent compound.

36. The use as claimed in claim 35, in which a 5 fluorescent conjugate as claimed in any one of claims 26 to 30 is used as a donor fluorescent compound in the presence of an acceptor fluorescent compound.

37. The use as claimed in claim 35, in fluorescence 10 microscopy, in flow cytometry, in fluorescence polarization or in fluorescence correlation.

38. The use of a conjugate as claimed in one of claims 26 to 30, as a contrast agent for optical imaging in 15 vivo.

39. A method for increasing the fluorescence intensity of a fluorophore attached to a carrier molecule, characterized in that a fluorescent entity as claimed in 20 one of claims 1 to 25 is used as a fluorophore.

40. A method for decreasing the phenomenon of aggregation at the surface of a carrier molecule attached to a fluorophore, characterized in that a fluorescent entity as claimed in one of claims 1 to 25 is used in 25 place of said fluorophore.

41. A method for increasing the quantum yield of a fluorophore attached to a carrier molecule, characterized in that a fluorescent entity as claimed in one of claims 30 1 to 25 is used as a fluorophore.